

WHAT IS CLAIMED IS:

1. A recombinant gelonin toxin other than the toxin of SEQ ID NO:1, which toxin comprises a core toxin region defined as amino acid residues 110-210 of SEQ ID NO:1.
- 5 2. The recombinant gelonin toxin of claim 1, comprising at least 10 contiguous amino acid residues of SEQ ID NO:1 in addition to the core toxin region.
3. The recombinant gelonin toxin of claim 2, comprising at least 20 contiguous amino acid residues of SEQ ID NO:1 in addition to the core toxin region.
- 10 4. The recombinant gelonin toxin of claim 3, comprising at least 30 contiguous amino acid residues of SEQ ID NO:1 in addition to the core toxin region.
5. The recombinant gelonin toxin of claim 4, comprising at least 50 contiguous amino acid residues of SEQ ID NO:1 in addition to the core toxin region.
- 15 6. The recombinant gelonin toxin of claim 1, wherein at least 10 amino acids of SEQ ID NO:1, other than amino acids from the core toxin region, are absent.
- 20 7. The recombinant gelonin toxin of claim 1, wherein at least 20 amino acids of SEQ ID NO:1, other than amino acids from the core toxin region, are absent.
8. The recombinant gelonin toxin of claim 1, wherein at least 30 amino acids of SEQ ID NO:1, other than amino acids from the core toxin region, are absent.
- 25 9. The recombinant gelonin toxin of claim 1, wherein at least 5 amino acids of SEQ ID NO:1, other than amino acids from the core toxin region, have been replaced.
10. The recombinant gelonin toxin of claim 9, wherein at least 10 amino acids of SEQ ID NO:1, other than amino acids from the core toxin region, have been replaced.
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11. The recombinant gelonin toxin of claim 10, wherein at least 20 amino acids of SEQ ID NO:1, other than amino acids from the core toxin region, have been replaced.
12. A proteinaceous compound comprising a recombinant gelonin toxin of claim 1
5 and a second polypeptide.
13. The proteinaceous compound of claim 12, wherein the second polypeptide is conjugated to the recombinant gelonin toxin.
- 10 14. The proteinaceous compound of claim 13, wherein the second polypeptide is conjugated to the recombinant gelonin toxin by a linker.
- 15 15. The proteinaceous compound of claim 12, wherein the second polypeptide and the recombinant gelonin toxin form a fusion protein.
- 16 16. The proteinaceous compound of claim 12, wherein the second polypeptide is an antibody.
- 20 17. The proteinaceous compound of claim 16, wherein the antibody comprises an antigen binding region.
- 25 18. The proteinaceous compound of claim 16, wherein the antibody is directed against a tumor antigen.
19. The proteinaceous compound of claim 12, wherein the second polypeptide has enzymatic activity.
20. A modified enzyme produced by a process comprising:
a) identifying one or more antigenic regions in the enzyme using an
30 antibody;

- b) removing one or more antigenic regions from the enzyme to form a modified enzyme; and
- c) determining that the modified enzyme has enzymatic activity.

5 21. The modified enzyme of claim 20, wherein determining comprises assaying the modified enzyme for enzymatic activity.

10 22. The modified enzyme of claim 20, further comprising replacing one or more antigenic regions with an amino acid region that is less antigenic than the replaced region or regions.

23. The modified enzyme of claim 22, wherein the less antigenic region or regions are identified in a protein database search for homologous regions.

15 24. The modified enzyme of claim 23, wherein the database is a human protein database.

20 25. The modified enzyme of claim 20, wherein the antigenic region is antigenic to a human.

26. The modified enzyme of claim 20, wherein the antibody is polyclonal.

27. The modified enzyme of claim 26, wherein the polyclonal antibody is from a human.

28. The modified enzyme of claim 20, wherein the enzyme is a plant toxin.

29. The modified enzyme of claim 28, wherein the plant toxin is gelonin.

30 30. The modified enzyme of claim 20, further comprising attaching a second polypeptide to the modified enzyme.

31. The modified enzyme of claim 30, wherein the second polypeptide is conjugated to the modified enzyme.

5 32. The modified enzyme of claim 30, wherein the second polypeptide and the modified enzyme form a fusion protein.

33. The enzyme of claim 30, wherein the second polypeptide is an antibody.

10 34. The enzyme of claim 30, wherein the second polypeptide is a toxin.

35. The enzyme of claim 30, wherein the second polypeptide is a second enzyme.

15 36. The enzyme of claim 30, wherein the second polypeptides promotes apoptosis.

37. A process for generating a modified protein that has reduced antigenicity comprising:

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- a) selecting a protein one desires to administer to a first subject;
 - b) identifying a region of the protein that is antigenic in the first subject using antiserum from either the first subject or a second subject of the same species as the first subject;
 - c) generating a modified protein in which the identified region is absent; and
 - d) confirming the modified protein has reduced antigenicity.

25 38. The process of claim 37, further comprising:

- d) screening a human protein database to identify a less antigenic region that has homology to the antigenic region of the protein;
- e) replacing the antigenic region with all or part of the identified region that is less antigenic to form a modified protein.

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39. The process of claim 38, wherein the replacing steps is accomplished recombinantly using a nucleic acid molecule encoding the modified protein.

5 40. The process of claim 37, wherein the modified protein is generated by removing the identified region.

41. The process of claim 37, wherein the absent region comprises at least 5 amino acid residues.

10 42. The process of claim 41, wherein the absent region comprises at least 10 amino acid residues.

15 43. The process of claim 42, wherein the absent region comprises at least 15 amino acid residues.

44. The process of claim 43, wherein the absent region comprises at least 20 amino acid residues.

20 45. The process of claim 44, wherein the absent region comprises at least 25 amino acid residues.

46. The process of claim 40, wherein the absent antigenic region is replaced with the same number of amino acid residues that are removed.

25 47. The process of claim 37, wherein the region is identified by ELISA assay.

48. The process of claim 37, wherein the subject is a mammal.

30 49. The process of claim 48, wherein the mammal is a human.

50. A humanized recombinant gelonin toxin having at least 3 amino acids from one or more of antigenic domains 1, 2, 3, or 4 replaced with amino acids less antigenic in a human than a recombinant gelonin toxin with the replaced amino acids.

5 51. The humanized recombinant gelonin toxin of claim 50, wherein at least 3 amino acids from antigenic domain 1 are replaced.

52. The humanized recombinant gelonin toxin of claim 50, wherein at least 3 amino acids from antigenic domain 2 are replaced.

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53. The humanized recombinant gelonin toxin of claim 50, wherein at least 3 amino acids from antigenic domain 3 are replaced.

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54. The humanized recombinant gelonin toxin of claim 50, wherein at least 3 amino acids from antigenic domain 4 are replaced.

55. The humanized recombinant gelonin toxin of claim 50, wherein at least 3 amino acids from at least 2 antigenic domains are replaced.

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56. The humanized recombinant gelonin toxin of claim 50, wherein at least 6 amino acids from one or more of antigenic domains 1-4 are replaced.

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57. A recombinant gelonin toxin produced by a process comprising:
a) identifying at least one region in a gelonin toxin that is antigenic in a mammal; and
b) replacing at least a portion of the antigenic region with a region less antigenic in the mammal.

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58. The recombinant gelonin toxin of claim 57, wherein the antigenic region identified in step a) is a recombinant gelonin toxin.

59. The recombinant gelonin toxin of claim 57, wherein the process further comprises comparing the identified antigenic region with mammalian amino acid sequences, whereby a region less antigenic in the mammal is identified.

5 60. The recombinant gelonin toxin of claim 57, wherein the process further comprises identifying a region less antigenic in the mammal.

61. The recombinant gelonin toxin of claim 60, wherein the mammal is a human.

10 62. A method of killing a cancer cell comprising providing to the cell an effective amount of an immunotoxin comprising a core toxin region of gelonin and single chain antibody that specifically targets the cancer cell.

15 63. The method of claim 62, wherein the immunotoxin comprises SEQ ID NO:1.

64. The method of claim 62, wherein the antibody targets a tumor antigen.

65. The method of claim 62, wherein the antibody is a mouse antibody.

20 66. The method of claim 62, wherein the cancer cell is a melanoma cell.

67. The method of claim 66, wherein the antibody comprises 9.2.27 or ZME-018.

25 68. The method of claim 66, wherein the immunotoxin is scfvMEL-2018 or scfvMEL-2025.

69. The method of claim 62, wherein the cell is in a patient.

30 70. The method of claim 69, wherein the immunotoxin is in a pharmaceutically acceptable composition.

71. The method of claim 62, wherein the immunotoxin is provided to the cell by administering to the cancer cell an expression construct capable of expressing the immunotoxin.

5 72. The method of claim 71, wherein the expression construct is a viral vector.

73. The method of claim 72, wherein the viral vector is an adenovirus vector, an adeno-associated virus vector, a hepatitis virus, a herpesvirus, a lentivirus, a retrovirus, or a vaccinia virus.

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74. A method of treating cancer in a patient comprising administering to the patient an effective amount of a composition comprising an immunotoxin comprising a core toxin region of gelonin and single chain antibody that specifically targets a cancer cell.

15 75. The method of claim 74, wherein the cancer is cancer of the prostate, lung, brain, skin, liver, breast, lymphoid, stomach, testicular, ovarian, pancreatic, bone, bone marrow, head and neck, cervical, esophagus, eye, gall bladder, kidney, adrenal glands, heart, colon, or blood.

20 76. The method of claim 74, wherein the immunotoxin comprises SEQ ID NO:1.

77. The method of claim 74, wherein the antibody targets a melanoma cell.

25 78. The method of claim 77, wherein the immunotoxin is scfvMEL-2018 or scfvMEL-2025.

79. A method for treating cancer in a patient comprising administering to the patient an effective amount of a composition comprising the humanized recombinant gelonin toxin of claim 50.

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80. The method of claim 79, wherein the composition further comprises an antibody.

81. The method of claim 80, wherein the antibody is humanized.
82. The method of claim 80, wherein the antibody is a single chain antibody.
- 5 83. The method of claim 80, wherein the antibody binds to an antigen on a cancer cell.
- 10 84. The method of claim 83, wherein the cancer cell is a bone, brain, breast, cervical, colon, glioma, gum, head and neck , kidney, leukemia, liver, lung, melanoma, ovarian, prostate stomach, or tongue cell.
85. The method of claim 84, wherein the cancer cell is a melanoma cell.
- 15 86. The method of claim 82, wherein the single chain antibody is 9.2.27 or ZME-018.
87. The method of claim 79, further comprising administering to the patient chemotherapy, radiotherapy, gene therapy or a second immunotherapy.
- 20 88. The method of claim 79, further comprising resecting a pre-cancerous or cancerous lesion or tumor from the patient.